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Microwave Assisted Synthesis, Antimicrobial and Anti-inflammatory Potential of Some Novel 1,2,4-triazole Derivatives

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ABSTRACT

A series of 1,2,4-triazole derivatives were synthesized under microwave irradiation and characterized by IR, NMR, and mass spectral data. Pathogenic microorganisms are causative agents for different types of serious infectious diseases. Despite advancements in medication, bacterial infections continue to be a growing problem in health care. As more and more bacteria become resistant to antibiotics used in therapy there is considerable interest in the development of new compounds with antimicrobial activity. Inflammation is a central part of the response to injury and infection in the immune system. It may become problematic if the inflammatory process continues for too long. External infections involving the skin and wound are the most frequent complications affecting humans and animals. The compounds containing a heterocyclic ring play an important role among organic compounds with biological activity used as drugs in human, veterinary medicine or as insecticides and pesticides in agriculture. The compounds were evaluated for antimicrobial and anti-inflammatory activity. The pharmacological evaluation of 1,2,4-triazole derivatives revealed that, among all the compounds screened compound code 2b showed leading antibacterial activity against the selected pathogenic strains of bacteria and compound code 2e were found to have promising anti-inflammatory activity.

Keywords: 1,2,4-triazole, microwave irradiation, antimicrobial screening, anti-inflammatory activity.

I. INTRODUCTION

Nitrogen containing heterocycles comprising of triazoles, benzothiazoles, benzimidazoles, indoles, etc. constitute an important scaffold in biological science and medicinal chemistry, and has fascinating applications in drug discovery and development[1]. In particular, the synthesis of 1,2,4-triazoles has attracted considerable attention during the last years. Several potent pharmacological properties such as anti-bacterial, antimicrobial, antioxidant, anticancer, and antitubercular of 1,2,4-triazole derivatives have

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been reported[2-4]. Microwave chemistry is the science of applying microwave radiation to chemical reactions. Microwave assisted organic synthesis has as a new "lead" in the organic synthesis[5-7]. This technique offers clean, simple, efficient, fast and economic for the synthesis of a number of organic molecules such reaction has new tool in the organic synthesis. Important advantage of this technology includes highly accelerated rate of the reaction time with an improvement in yield and quality of product[8]. Experiments have proved that microwave method, in comparison with the Soxhlet extraction, use a lesser volume of solvent and sample and perform extraction at a much faster rate were previously reported for various plant extractions. Due to failure of ADME so it necessary to perform docking studies before pharmacological activity for triazole molecules. An outbreak of coronavirus disease (COVID-19) caused by the novel severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) raises an unparalleled challenge in the discovery of appropriate drugs for prevention and treatment. Given the rapid pace of scientific research and clinical data produced by the large number of people quickly infected with SARS-CoV-2, clinicians need reliable proof of successful medical care for this infection as in this pandemic in-silico docking studies of 1,2,4-triazole have shown good results. The chemical modification of drug delivery system for protein and peptide drugs is important in improving both enzymatic stability and membrane permeations can help to have good biological activity from any heterocyclic compound modification. Someday soon, you might be making your own medicines at home. That's because researchers have tailored a 3D printer to synthesize pharmaceuticals and other chemicals from simple, widely available starting compounds fed into a series[9-22]. The search for newer sources of antibiotics is a global challenge pre-occupying research institutions, pharmaceutical companies, and academia, since many infectious agents are becoming resistant to synthetic drugs. Emergence of resistant strains of pathogenic microorganism has also continued to pose a major health concern about the efficacy of several drugs, most importantly antibiotics in current use. At present lot of drugs are accessible for curing the microbial infection but most of them is becoming abortive due to antimicrobial resistance by the micro-organisms. So there is an immense need for the discovery of novel antimicrobial agents to overcome the antimicrobial resistance and side effects[23-25]. Inflammation is a complex biological response of vascular tissue to harmful stimuli, pathogens, irritants characterized by redness, warmth, swelling and pain Prolonged inflammation leads to the rheumatoid arthritis, atherosclerosis, hay fever, ischemic heart diseases etc and inflammation is a common manifestation of infectious diseases like leprosy, tuberculosis, asthma, inflammatory bowel syndrome, nephritis, vascularitis, celiac diseases and auto-immune diseases etc. In this research article, we have reported the microwave assisted synthesis of new 1,2,4-triazole derivatives. These compounds were tested for antimicrobial and anti-inflammatory activity[26].

II. MATERIAL AND METHODS

All chemicals and solvents were procured from commercial sources, purified and dried using standard procedures from literature whenever required the regents were purchased from S. D fine, research laboratory and merck laboratory, Mumbai. The melting points of synthesized compound were determined by open capillary tube method and are uncorrected. Thin layer chromatography was used confirmation of reaction and the purity of the intermediate and the final compounds by applying a single spot on TLC plate (silica gel G) using various solvents such as butanol, chloroform, water system. TLC plates were visualized under iodine chamber. IR spectra were recorded on FTIR, NMR spectra were

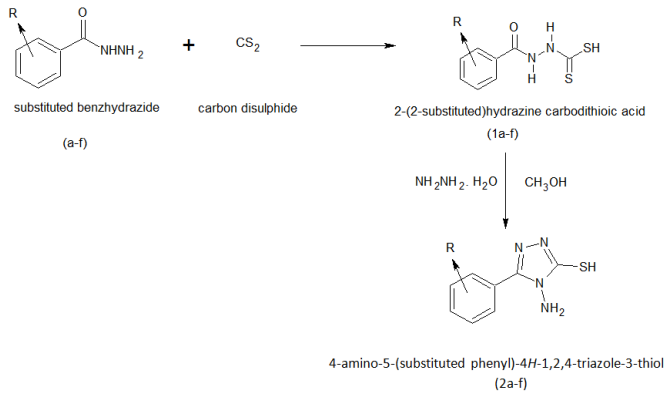
performed in DMSO solution using Bruker 300 MHz and their chemical shift are reported in δ unit with respect to TMS as internal standard. Mass spectra were recorded on Pe sciex (model no. API 2000) software analyst 1.4.2 mode: Q1MS Q1/AUTO INJECTION from diya lab, airoli, Mumbai. General preocedure for synthesis of 1,2,4-triazole derivatives are as follows:

General method for the Synthesis of 2-(2-substituted)hydrazine carbodithioic acid

Mixture of substituted benzhydrazide (2g) and carbon disulphide (1.5ml) was irradiated for 15 min at 340 watt under microwave. The reaction was monitored by TLC using chloroform: methanol (9:1) as mobile phase.

General method for the Synthesis of 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol

A product of 2-(2-substituted)hydrazine carbodithioic acid was added in hydrazine hydrate (2ml) and methanol (10ml) and mixture was irradiated for 20 min at 340 watt under microwave. The reaction was monitored by TLC using butane: chloroform: water (7:2:1) as mobile phase. The solid product was washed with water and recrystallized with methanol.



Scheme 1: Synthetic route for the preparation of the title compound (2a-f)

Analytical Data of Novel 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol Derivatives:

2a. 4-amino-5-(4-nitrophenyl)-4*H*-1,2,4-triazole-3-thiol

Yield 72%; m.p 150-152⁰C; IR (KBr, cm-¹) 1338.12 (AR-NO_2), 1651.54 (C=C), 2253.32 (C-C), 2485.11 (S-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.20 (s, 1H), 5.59-5.68 (d, 2H, ArH), 7.40-7.47 (q, 4H, ArH); mass m/z (M+) 237.3.

2b. 4-amino-5-(chloromethyl)-4*H*-1,2,4-triazole-3-thiol

Yield 67%; m.p 142-144⁰C; IR (KBr, cm-¹) 669.12 (C-Cl), 1630.72 (C=C), 2490.72 (S-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.81 (s, 1H), 5.35-5.40 (d, 2H, ArH); mass m/z (M+) 164.7.

2c. 4-amino-5-(chlorosulfanyl)-4*H*-1,2,4-triazole-3-thiol

Yield 63%; m.p 147-149⁰C; IR (KBr, cm-¹) 752.81 (C-S), 2460.31 (S-H), 3452.18 (N-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.28 (s, 1H), 5.58-5.65 (d, 2H, ArH); mass m/z (M+) 182.4.

2d. 4-amino-5-[4-(trifluoromethyl)phenyl]-4*H*-1,2,4-triazole-3-thiol

Yield 83%; m.p 155-157⁰C; IR (KBr, cm-¹) 1671.42 (C=C), 2240.18 (C-C), 2443.72 (S-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.40 (s, 1H), 5.49-5.58 (d, 2H, ArH), 7.39-7.48 (q, 4H, ArH); mass m/z (M+) 260.3.

2e. 4-amino-5-(2,4-dimethoxyphenyl)-4*H*-1,2,4-triazole-3-thiol

Yield 78%; m.p 152-154⁰C; IR (KBr, cm-¹) 1679.35 (C=C), 2253.87 (C-C), 2471.19 (S-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.51 (s, 1H), 5.49-5.57 (d, 2H, ArH), 7.41-7.58 (q, 4H, d, ArH); mass m/z (M+) 252.3.

2f. 4-amino-5-[4-(dimethylamino)phenyl]-4*H*-1,2,4-triazole-3-thiol

Yield 87%; m.p 167-169⁰C; IR (KBr, cm-¹) 1651.49 (C=C), 2817.12 (N-CH_3), 2244.52 (C-C), 2469.11 (S-H), 1H NMR (DMSO, 300MHz, ppm): δ 2.40 (s, 1H),

3.25-3.21 (t, 2H, ArH), 5.31-5.38 (d, 2H, ArH), 7.62-7.69 (q, 4H, ArH); mass m/z (M+) 235.4.

BIOLOGICAL EVALUATION:

A. Antimicrobial screening:

Chemicals

All chemicals and solvent where procured from commercial sources, purified and sterilize using standard procedure from literature whenever required.

1. Dilution of compound

All the synthesized compound where dissolved in dimethyl sulphoxide [DMSO] so as to get concentration of 100µg/ml and 200µg/ml, and standard drug ciprofloxacin in DMSO as a concentration of 200µg/ml.

2. Sterilization of equipment and the chemicals

MacConkey agar, Nutrient agar medium [NO11], Normal saline solution where sterilized in autoclave. At 15 Ibs pressure [12IC] for 150 min. Petri plates, Whatman filter paper, descant cotton swabs where sterilized in oven at 160^{0}C for 2 hrs.

3. Preparation of MacConkey agar slant

MacConkey agar 206 mg was dissolved in 4ml of distilled water, boiled and poured test tube then plugged with cotton and sterilize in autoclave as 15 Ibs pressure 121°C for 15 min. After sterilization the tubes containing the MacConkeys agar were kept in inclined position from 30 min. Then on the surface of slants pure culture staphylococcus aureus where streaked in aseptic condition and incubated and 37°C for the 24 hours.

4. Preparation of nutrient agar medium slant

Nutrient agar medium 112 mg and agar powder 100 mg was dissolved in 4 ml distilled water, boiled and then poured in test tube then plugged with cotton and sterilized in autoclave at 15 Ibs pressure (121°C) for 15 min. After the sterilization the tubes containing the nutrient agar medium were kept in inclined position for 30 min. Then on the surface of slants pure culture of *E.coli* were streaked in aseptic condition and incubated at 37°C for 24 hours.

5. Preparation of suspension of test bacteria:

Using the 24 hours old growth of test bacteria from the slant, suspension of bacteria was made separately in sterile normal saline solution (0.85% NaCl in distilled water) in aseptic condition, to get moderate turbidity. The turbidity of each suspension was compared adjusted with the turbidity of the solution resulting by mixing 0.5 ml of 1.175% of barium chloride and 99.5 mi of 36 N of H_2SO_4.

Method: Disc Diffusion Method

1. Preparation of culture media for antibacterial sensitivity test

MacConkey agar (50ml) and nutrient agar (100ml) was prepared as per the procedure given for preparation of slants respectively. Then it was sterilized in autoclave at 15lbs pressure (121^{0}C) for 15 min. after sterilization the media was cooled up to 45^{0}C, poured 20-25 ml in sterile Petri plates in aseptic condition and allowed to solidify.

2. Inoculation of suspension of bacteria on culture media

Sterile, non toxic swab were dipped into the standardized inoculums and then the entire agar surface of the plate was streaked with the swab three times, turning the plate at 60 angles between streaking. Then the streaked inoculum was allowed to dry for 5-15 min with lid. Sterile Whatman paper disc dipped separately into the solutions containing synthesized drug (100µg/ml and 200µg/ml) and

standard drug ciprofloxacin (100µg/ml and 200µg/ml) in aseptic condition with the help of sterile forceps and placed on the surface of inoculated culture media after which the plates were kept in refrigeration for 30min. for the diffusion of the compound from the paper disc into the culture media. After 30 min. The plates were incubated at 37^{0}C for 24hrs. All the synthesized compounds (2a-f) were observed for antibacterial activity. Observation was recorded in tables by measuring the zone of inhibition in millimeters[27].

B. Anti-inflammatory Evaluation

Method: Protein denaturation method

The mixture (5ml) consisted of 0.2ml of egg albumin (from fresh hen's egg), 2.8ml of Phosphate buffered solution (PBS, pH 6.4) and 2ml of varying concentration of test samples so that final concentration become 50µg/ml and 100µg/ml. Similar volume of DMSO served as control. Then the mixtures were incubated at (37^{0}C ± 2) for 15 min. and then heated at 70^{0}C for 5min. After cooling, their absorbance was measured at 660nm (JASCO UV spectrophotometer) by using vehicle as blank and their viscosity was determined by using ostwald viscometer. Diclofenac at the final concentration of 50µg/ml and 100µg/ml was used as reference drug and treated similarly for determination of absorbance and viscosity.

The % inhibition of protein denaturation was calculated by using the following formula[28-29].

$$\% \text{ inhibition protein denaturation} = \frac{\text{Absorbance of control - Absorbance of test}}{\text{Absorbance of control}}$$

III. RESULT AND DISCUSSION

Chemistry

In first step mixture of substituted benzhydrazide and carbon disulphide was irradiated for 15 min at 340 watt under microwave. The reaction was monitored by TLC using chloroform: methanol (9:1) as mobile phase. A product of 2-(2-substituted)hydrazine carbodithioic acid was added in hydrazine hydrate and methanol and mixture was irradiated for 20 min at 340 watt under microwave. The reaction was monitored by TLC using butane: chloroform: water (7:2:1) as mobile phase. The solid product was washed with water and recrystallized with methanol. The reaction sequence is shown in Scheme 1. Microwave assisted synthesis is faster, better and safer green chemistry approach for the traditional reactions. The time taken for the synthesis of 1,2,4-triazole is drastically reduced by the microwave assisted synthesis. This technique offers clean, simple, efficient, fast and economic for the synthesis of a number of organic molecules such reaction has new tool in the organic synthesis and highly accelerated rate of the reaction time with an improvement in yield and quality of product. The IR, NMR and mass spectra are fully consistent with the structure.

Antimicrobial activity:

Antibacterial activity of the newly synthesized compounds (2a-f) was evaluated by the disc diffusion method against Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus strains of bacteria. Compound code 2b were found to be highly active against all the tested strains of bacteria showing the broadest spectrum of antibacterial activity when compared with standard drug ciprofloxacin.

Table 1: Antibacterial screening result of synthesized compounds (2a-f) measuring the zone of inhibition in millimeter

Sr. No	Compound code	Diameter of zone of inhibition (mm)					
		E. coli		*P. aeruginosa*		*S. aureus*	
		100µg/ml	200µg/ml	100µg/ml	200µg/ml	100µg/ml	200µg/ml
1	2a	12	15	9	13	16	18
2	2b	19	27	16	19	22	25
3	2c	10	13	8	14	12	15
4	2d	14	19	11	12	17	19
5	2e	8	11	10	13	10	14
6	2f	16	21	7	11	8	12
7	Ciprofloxacin	22	26	18	20	21	23

Anti-inflammatory activity:

In present study in-vitro results confirmed anti-inflammatory activity of new series of 1,2,4-triazole evaluated by denaturation of proteins which is a well documented cause of inflammation. Several anti-inflammatory drugs shown dose dependent ability to inhibit thermally induced protein denaturation. Ability of 1,2,4-triazole is to bring down thermal denaturation of protein is possibly a contributing factor for its anti-inflammatory activity. The data of our studies suggests that compound code 2e shows significant anti-inflammatory activity.

Table 2: Anti-inflammatory activity of synthesized compounds measuring inhibition of protein denaturation

Sr. no	Compound code	% inhibition of protein denaturation		Viscosity (cps)	
		50 µg/ml	100 µg/ml	50 µg/ml	100 µg/ml
1	2a	61.35	63.29	0.44	0.48
2	2b	57.73	71.65	0.40	0.43
3	2c	61.67	63.41	0.42	0.45
4	2d	69.31	70.42	0.48	0.50
5	2e	75.12	78.16	0.52	0.56
6	2f	62.70	67.19	0.47	0.49
7	Diclofenac	78.14	82.52	0.50	0.54

IV. CONCLUSION

A series of 4-amino-5-(substituted phenyl)-4H-1,2,4-triazole-3-thiol were synthesized by microwave method and characterized by IR, NMR and mass spectra. All newly synthesized were screened for antibacterial activity. Among them compound code 2b showed excellent antibacterial activity. The results of anti-inflammatory activity highlighted that tested compound code 2e exhibited significant activity by protein denaturation method.

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